Am J Hum Genet 32:432–444, 1980

Expected and Observed Proportion of Subjects Excluded from Paternity by Blood Phenotypes of a Child and Its Mother in a Sample of 171 Families

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SUMMARY

The proportion of exclusion for a given mother-child pair is the proportion of males excluded from the paternity of this child of a known mother and may be calculated given both the child's and mother's phenotypes and the population gene frequencies. Its expected value in the population is equal to the probability of exclusion, which expresses a laboratory's capability to exclude from paternity nonbiological fathers.

In a sample of 171 families examined for 20 genetic systems at the National Blood Group Reference Laboratory, 25 exclusions of putative fathers were detected. The ranking by efficiency of the systems used in these exclusions fits the "expectation of their efficiency," and the average proportion of males excluded by the child's and mother's phenotypes is not different from the expected proportion. Additionally, the repetition of exclusions in an incompatible putative father-mother-child trio is not dependent on the overall proportion of males excluded by the mother and the child, but rather on some high values of the proportion of excluded men in some specific systems.

Here, formulas and some factors modifying these parameters as well as a more efficient sequence of examinations to exclude paternity than has previously been used are given. Using this sequence, laboratories which carry out several analyses per day can work by levels of five examinations at a time, done in a particular order, to obtain a rather rapid exclusion of certain families.

Received May 5, 1979; revised August 17, 1979.

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INTRODUCTION

Presently, paternity can be excluded when there is a clear incompatibility between the blood phenotypes of the putative father and those of the child and its mother. The importance of these paternity determination studies is twofold: first, some European countries require paternity testing for forensic purposes; and second, accurate genetic studies require definite paternity determination.

The evaluation of the probability of paternity exclusion of a falsely accused putative father and the distribution of the number of exclusions have been presented [1, 2]. This present study compares the theoretical results of paternity exclusion expected based on 20 immunogenetic systems with the actual results obtained from a sample of 171 families. This study is, thus, based both on population genetics (i.e., on Mendelian laws and probability axioms) and on immunogenetic methods practiced in a specialized laboratory.

DEFINITION

There are two major paternity exclusion rules in current use*: the first is invoked when the child possesses an allele which is absent from both the mother and the putative father; the second, when the child lacks an allele which he or she should possess based on the genotypes of the mother and the putative father. A third rule may also be defined [3] for the case in which a sibship is shown to be incompatible with the putative father's phenotype, although each child's phenotype, analyzed by itself, is compatible with this paternal phenotype. Race and Sanger [4] cite the case of a sibship in which the segregation of *MNSs* alleles needed at least three different fathers.

Proportion of Excluded Males (P_{ex}) and Probability of Exclusion (P)

Let us examine the phenotypes of a given mother-child pair for polymorphic systems which obey simple Mendelian segregation and for which gene frequencies estimates are known. It is possible, then, to calculate the proportion of excluded males (P_{ex}) for males chosen at random from the population. This calculation expresses the chance that a male who is not the biological father will actually be excluded with respect to this particular mother-child pair. Its expected value is the probability of exclusion (P) which concerns a man taken at random and a mother-child pair, also taken at random, from the population. P expresses the laboratory's capability to exclude a falsely accused man.

METHODS

Determination of P Based on the Genetic Frequency of Marker Systems

The probability of exclusion (P) or the expected value of the proportion of excluded men (P_{ex}) may be expressed for each genetic system in terms of the estimated population gene frequencies at that particular locus [1], assuming that the population is panmictic (i.e., that the choice of mate is random with respect to the genetic system) and that there is no selection or mutation. Hanset gives the probability of exclusion (P) for a locus with two, three, or n alleles [5]. For n

* European forensic experts firmly insist on observing a first rule of exclusion because it is much more reliable than the second one.

alleles, algorithmic methods have been shown to be preferable to arithmetical methods, and such an algorithmic method has been developed for computer usage [6].

Let N genetic systems be examined, and let p_i be the probability of exclusion calculated for the ith system. The overall probability of exclusion can be expressed [1] as

$$P = 1 - \prod_{i=1}^{N}(1 - p_i) \ . \tag{1}$$

Calculation of P_{ex} for a Mother-Child Pair

Similarly, we can write

$$P_{ex} = 1 - \prod_{i=1}^{N}(1 - p'_i) \ , \tag{2}$$

where p_i' is the proportion of subjects excluded for the ith system. A computer program has been developed for such an algorithm [6].

Several factors modify the probability of exclusion (P) and the proportion of subjects excluded (P_{ex}) for a given mother-child pair under the hypothesis of panmixia and in the absence of consanguinity between the biological parents.

Dominance of one character over another. In immunology, for example, the absence of an antiserum causes certain genotypes to be indeterminate. For such phenotypes, the allele a parent transmits or a child receives cannot be identified. The Kidd system is a case in point. In Western European populations, the single antiserum anti-Jk^a excludes 2.87% of males; use of both anti-Jk^a and anti-Jk^b excludes 18.74%. For the ABO system, 16.81% of males are excluded with anti-A, anti-A_1, and anti-B. Were it possible to recognize all of the genotypes at this locus, 28.62% of males could be excluded.

Gene frequencies. For n codominant alleles of frequencies f_i, the probability of exclusion (P) is maximum for $f_i = 1/n$ (i.e., when the frequencies of the various alleles are equal in a polymorphic system). In addition, as the number of alleles increases, so does P.

A genetic locus carrying several codominant polymorphic alleles, each of significant frequency, would thus be particularly efficient for paternity exclusion; this is why the enzymatic systems, such as acid phosphatase (AcP), are so informative. Also, the numerous genetic systems carrying one or more rare alleles (i.e., with a frequency of less than .005), if expressed in single dose, can considerably increase the proportion of subjects excluded for a mother-child pair. The rare allele present in the child, but absent in the mother, can only come from the biological father, and this excludes a large proportion of the population.

If the HLA system so frequently reveals nonpaternity, it is because this system satisfies several of the conditions stated above, as it is comprised of numerous alleles of both high and low frequencies.

The Number of Marker Systems Examined for the Mother-Child Pair

Obviously, the proportion of subjects excluded increases with the number of systems examined, as is clear from the formula for the exclusion probability (P) for N marker systems, each giving a probability p_i of exclusion ($i = 1, \ldots, N$), as in equation (1), and from the formula for calculating the total proportion of exclusion for a mother-child pair as in equation (2).

Is it preferable to examine a large number of biallelic systems or a smaller number of multiallelic systems to obtain a high value of P for a population and of P_{ex} for a specific mother-child pair? Hanset [5] considered these alternatives and concluded that the use of multiallelic systems is the more efficient. This has been observed using the HLA system.

The Effect of Parental Consanguinity on P_{ex} by the Mother-Child Pair

We have assumed above that the child's mother, its biological father, and the putative father all came from a panmictic population and that there was no consanguineous relationship between

any pair of them. The assumption that in panmictic populations marriages are random is useful and necessary in population genetics. In practice, however, the effective choice of a mate is often limited by sociological and geographical barriers, and if subjects belong to the same isolate, they, consequently, are more or less consanguineous. A pair of subjects, therefore, could have received from a common ancestor two genes "identical by descent" [7].

Both geneticist and magistrate are often presented with a paternity case involving a child whose mother and father are closely related—they may be first cousins, brother and sister, or father and daughter. When parental consanguinity is involved, the probability of exclusion (P) can be altered [8]; that is, in genetic research, the probability of rejecting an invalid family file and, in forensic practice, the chances that a falsely accused male is cleared of the accusation of paternity are both decreased. The geneticist, unaware of possible consanguinity, who contents him- or herself with observing that for N systems examined he or she has found no exclusion, risks an error. The calculation of the probability of exclusion, P, of a "random" individual based on the series of immunogenetic systems used plus the calculation of the proportion of subjects excluded (P_{ex}) by a mother and her child makes it possible to verify the results [9, 10].

MATERIAL

The Blood Group Research Unit and National Blood Group Reference Laboratory examined 20 genetic marker systems on 171 families. The test procedure was rigorously adhered to, regardless of the family's mode of ascertainment. Each blood sample was examined by the same methods with the same antisera (i.e., anti-s for MNSs was used, and anti-c or e for Rhesus (Rh) was used systematically for all subjects—not only for S(+), C(+), or E(+) subjects). Tests were performed in duplicate and with two different antisera, and the results were interpreted by two technicians. Certain families also have been studied for the polymorphism of esterase D (ESD) and for the HLA system introduced into the laboratory examination routine while the present study was already in progress (1975).

All 171 families, of which 76 had one child and 95 between two and 10 children, were examined according to the established procedure. The families studied came from two sources—they had been sent either for forensic reasons (to establish or refute paternity) or for genetic evaluation (i.e., families where at least one child presented a congenital or hereditary anomaly or control families volunteering for systematic examination). They were mostly French, coming from Paris or, occasionally, from the provinces.

Certain biases may have been introduced into the sample, particularly respecting the genetic propositi; some came to the laboratory because a child had an anomaly and, therefore, cannot be considered as forming part of a random sample. Additionally, a preselection may have taken place as a previous exclusion in the ABO or Rh system may have eliminated some families from the beginning. Also, some families were selected through blood donors and were, thus, not chosen at random, while in other cases, selection was made on the basis of the segregation of a rare *ABO* allele of particular interest in genetic research.

The calculations of P and P_{ex} were performed on the basis of Western European gene frequencies published by Race and Sanger [4], as well as on estimations from the Centre National de Transfusion Sanguine (CNTS) laboratory data [11–13]. The existence of a silent Duffy allele having a frequency of .03 was assumed, but no such assumption was made for the Kidd system.

RESULTS

Table 1 shows the "historic" (i.e., chronological—ABO first, etc.) sequence of examination of the 21 polymorphic systems studied by Group Research in 1975. The value of P for each of these systems is indicated in the first column, and, additionally, for each step in the examination sequence, in the second column. The overall probability of exclusion is .9440 (ESD not included). To minimize the cost, an attempt was made to optimize the efficiency of the sequence of examinations. *Optimization of*

TABLE 1

HISTORIC SEQUENCE OF IMMUNOGENETIC TESTS

Genetic system	P^*	$P\dagger$	Alleles sought
ABO	.1671	.1671	*A1, A2, B*
Rh	.2906	.4091	$CC^w cDEe$
MNSs	.3139	.5946	*MNSs*
P	.0298	.6067	*P1*
K	.0416	.6230	*K k Kpa Kpb*
Fy	.0706	.6497	$Fy^a Fy^b$
Jk	.1872	.7152	$Jk^a Jk^b$
Lu	.0274	.7230	$Lu^a Lu^b$
Gm	.2298	.7867	*Gm1 Gm12 Gm45*
Inv	.0587	.7992	*Inv1*
Gc	.1583	.8310	···
Hp	.1813	.8616	···
C_3	.1419	.9813	···
Tf	.0079	.8822	···
AcP	.2350	.9099	···
PGM_1	.1565	.9240	···
AK	.0384	.9266	···
ADA	.0386	.9294	···
6PGD	.0229	.9311	···
GPT	.1873	.9440	···
ESD	.0889	.9490	···

* Exclusion probability of man (chosen at random) by system.
† Exclusion probability of man (chosen at random) for each step of examination sequence.

efficiency is defined as determining a sequence of examinations which gives the maximum amount of information in the minimum number of steps. An *optimally efficient sequence* is that which has the best chance of excluding the paternity of a falsely accused father with the minimum number of analyses.

Table 2 regroups the genetic systems in decreasing order of efficiency, and this, called the "efficiency sequence," shows that the ABO system (eighth row), with four alleles and dominance relationships, is half as efficient as the MNSs (first row), which has four haplotypes and where the only indeterminacy is that of the phase of the double heterozygote MNSs. Note that the enzymatic groups are at the head of the efficiency sequence, but at the end of the historic sequence. Currently well-established systems in laboratory practice, such as Kell Cellano, Lutheran, and P are, in fact, of little use in decisions concerning paternity.

The HLA system was not included because a homogeneous nomenclature for the alleles was not yet available, and cross-reactions sometimes modified the results. With the large number of HLA alleles recognized at each locus, however, this system would otherwise lead the efficiency sequence. The "transfusion sequence," which is used in blood bank practice and is limited to ABO and Rh, as well as K, Fy^a, Jk^a, and S antigens, is not sufficient to detect paternity (51% capability of exclusion).

Probability of exclusion (P) is an index which has the disadvantage of varying as a function of gene frequencies from one population to another, but has the advantage of being independent of the laboratory carrying out the procedure. (This advantage can, unfortunately, be nullified by the slightest lack of competence in the laboratory, such as failure to identify a rare allele or even a typing error.)

TABLE 2

EFFICIENCY SEQUENCE OF IMMUNOGENETIC EXAMINATIONS

Genetic System	P	P
MNSs	.3139	.3139
Rh	.2906	.5133
AcP	.2350	.6277
Gm	.2298	.7132
GPT	.1873	.7669
Jk	.1872	.8106
Hp	.1813	.8449
ABO	.1671	.8708
Gc	.1583	.8913
PGM_1	.1565	.9083
C_3	.1419	.9213
ESD	.0889	...*
Fy	.0706	.9269
Inv	.0587	.9311
K	.0416	.9340
ADA	.0386	.9366
AK	.0344	.9387
P	.0298	.9406
Lu	.0274	.9422
6PGD	.0229	.9435
Tf	.0079	.9440

NOTE.—For explanation of columns 1 and 2, see table 1 footnotes.
* ESD omitted.

For the Western European populations, P is practically constant, and the efficiency sequence of table 2 is valid. One can work at several levels when there are many samples to be examined: the first, up to and including ABO; then four steps further to ESD, etc. Technical constraints may lead to the modification of the proposed ranking of examinations. For example, ESD (12th row), ADA (16th row), AK (17th row), and 6PGD (20th row) may be tested simultaneously (E. Robson, personal communication, 1979). But advances in technology and differences in routine procedures used in various laboratories may make general rules of processing difficult, each laboratory being aware of its own management possibilities. Moreover, ABO and Rh, because of their clinical importance, may be placed at the head of the sequence. It is also possible, in the case of a family file in genetic research, to decide to limit the analysis to N systems (hence, reaching only a limited value of P) when it is desirable to eliminate quickly the most flagrant exclusions [14].

In conclusion, the efficiency sequence indicates the order in which the various marker systems should be examined if information on blood relationships within the trio is needed in the minimum time. This sequence permits the mathematical expectation of the proportion of those excluded (P_{ex}) either to be fixed a priori (and, thus, to determine the number of marker systems that must be examined) or to proceed by levels of examination.

OBSERVATIONS ON THE SAMPLE OF 171 FAMILIES

Twenty-five paternity exclusions were found, among which 13 were for single-child families (T's)—11 forensic and 2 genetic cases. The other 12 were for children from 12

sibships (F's) analyzed for genetic purposes. Curiously, each of these 12 children could have been excluded solely by examination of the phenotypes of the putative father and mother, without considering the phenotype of the sibs to determine a parental genotype. Thus, these 12 excluded trios (F1–F12) can be grouped together with the 13 exclusions relating to T's (T1–T13). This gives a sample of 25 children incompatible with the putative father.

There remained 61 T's and 75 F's for whom no exclusion was observed. Only the 61 T's will be analyzed here, because the other father-mother-child trios were not genetically independent.

Table 3 shows the number of exclusions observed in the excluded trios. Tables 4 and 5 show the number of exclusions observed for each system.

The T4 trio already excluded three times probably presents an additional exclusion in the Duffy system (father Fy[a+b−], child Fy[a−b+]), despite the presumed existence of a silent allele. Similarly, for the child belonging to the F12 trio, a single exclusion according to the second rule by the Duffy system (Father Fy[a−b+], mother Fy[a+b−], child Fy[a+b−]) should probably be added. The most probable genotypes for the parents were Fy^bFy^b and Fy^aFy^a, respectively. Their nine other children are all Fy[a+b+], which gives .9995 a posteriori probability for these parental genotypes. The T1 trio, already excluded four times, is further excluded by ESD (presumed father 2, child and mother 1). The eight trios examined for HLA were also excluded by this system and, except for two cases, by the first rule.

Birth Rank of the Excluded Children

Five of the excluded children belonging to a sibship are the eldest of the sibship, and this has been a common experience in our laboratory in families not belonging to the present series. It is probably significant that six of the other 12 were the last of their sibship.

Number of Exclusions Observed and Expected for Each System

For the 25 trios (T1 to T13 trios studied together, F1 to F12 trios isolated from the sibships), the nonpaternity of the putative father is certain. Thus, a sample has been

TABLE 3

NO. EXCLUSIONS OBSERVED IN T AND F EXCLUDED TRIOS

Total no. systems detecting exclusion	No. first rule exclusions	T trios	F trios	Total trios
4	2	0	2	2
4	1	2	0	2
3	2	0	2	2
3	1	2	1	3
2	2	3	1	4
2	1	5	1	6
2	0	0	1	1
1	1	1	4	5

NOTE.—For example (first line of table): the exclusion of paternity was observed four times in the same trio (and two times according to the first rule of exclusion) in 0 T trios and in 2 F trios; total = 2 trios.

TABLE 4

OBSERVED AND EXPECTED NO. EXCLUSIONS ON TRIOS

Genetic system	25 EXCLUDED TRIOS			13 T EXCLUDED TRIOS			12 F EXCLUDED TRIOS		
	O	E	χ^2	O	E	χ^2	O	E	χ^2
MNSs	10	7.85	0.86	4	4.08	0.002	6	3.77	1.93
Rh	5	7.26	0.99	3	3.78	0.23	2	3.49	0.89
AcP	5	5.87	0.17	2	3.06	1.62	3	2.82	0.01
Gm	5	5.74	0.12	2	2.99	0.42	3	2.76	0.03
GPT	5	4.68	0.03	3	2.43	0.16	2	2.25	0.03
Jk	5	4.68	0.03	4	2.43	1.24	1	2.25	0.85
Hp	1	4.53	3.36	1	2.36	0.95	0	2.18	2.66
ABO	1	4.18	2.90	1	2.17	0.76	0	2.00	2.61
Gc	2	3.96	1.15	1	2.06	0.65	1	1.90	0.51
PGM_1	4	3.91	0.002	1	2.03	0.62	3	1.88	0.79
C_3	2	3.55	0.79	1	1.84	0.45	1	1.70	0.34
Fy	3	1.90	0.69	1	0.99	0.0002	2	0.91	1.40
Inv	1	1.47	0.16	1	0.76	0.08	0	0.70	0.75
K	2	1.04	0.92	1	0.54	0.41	1	0.50	0.52
ADA	2	0.96	...	2	0.50	...	0	0.46	...
AK	1	0.86	...	1	0.45	...	0	0.41	...
P	2	0.74	...	1	0.39	...	1	0.36	...
Lu	1	0.68	...	1	0.36	...	0	0.33	...
6PGD	1	0.57	...	0	0.30	...	1	0.27	...
Tf	0	0.20	...	0	0.10	...	0	0.09	...

NOTE.—Expected no. (E) is calculated from the probability of exclusion (P) by each of the systems. χ^2 values are for fit between E and observed (O) nos.

constructed on the basis of 20 marker systems, which consists of 25 putative but not biological fathers. From this sample, the efficiency of these systems in the demonstration of nonpaternity (i.e., the probability of exclusion of a male who is not the biological father, p_i) can be calculated on the basis of the population gene frequencies.

In a sample of 25 trios, the expected number of exclusions for the ith system is equal to the product: $p_i \times 25$ (or 13 and 12 for the T and F groups, respectively). Table 4 gives the expected numbers and the value of χ^2 for fit between the expected and the observed numbers. For 1 df, the deviation is significant if $\chi^2 \geq 3.84$, a value never reached in these results. Nevertheless, two values are underlined which were rather high for χ^2 ($P < .10$), corresponding to a deficiency in exclusions revealed by ABO and Hp, particularly among the putative fathers taken from the sibship sample. The χ^2 test was not calculated for the last six lines of the table because the expected number was too low.

The absence of exclusion by the ABO system in genetic sibships can be explained by the two biases affecting the sample: a preselection that eliminates exclusions in the blood group most often analyzed (ABO) and a specific ascertainment of families of interest at the *ABO* locus, which is a topic of special research at our laboratory. For the 12 genetic sibships revealing exclusion, one (F7) has a putative father of genotype A_{el} [15], and in another (F9), the child is A_{int}.

As for the absence of exclusion by the Hp system in the same sibships in the 12 mother-child pairs, five were of mother 2.1-child 2.1 type (higher than the expected probability, 0.24, of this type of pair), which is incompatible with the demonstration of

TABLE 5

EXPECTED P AND OBSERVED P_{ex} FOR SAMPLE OF 171 FAMILIES

GENETIC SYSTEM	EXPECTED P		OBSERVED P_{ex}									
			61 non-excluded trios		25 excluded trios			13 T excluded trios			12 F excluded trios	
		R		R		R	N		R	N		R	N
MNSs	.3139	1	.3279	1	.3319	1	10	.3358	1	4	.3278	3	6
Rh	.2906	2	.2509	3	.2464	3	5	.3129	2	3	.1743	8	2
AcP	.2350	3	.2147	4	.2740	2	5	.2162	4	2	.3366	1	3
Gm	.2298	4	.2674	2	.2364	4	5	.2007	6	2	.2752	4	3
GPT	.1873	5	.1726	6	.1927	7	5	.2274	3	3	.1550	9	2
Jk	.1872	6	.2059	5	.2188	6	5	.2104	5	4	.2279	5	1
Hp	.1813	7	.1707	7	.1291	10	1	.1157	11	1	.1437	10	0
ABO	.1671	8	.1520	9	.2194	5	1	.1143	12	1	.3307	2	0
Gc	.1583	9	.1341	10	.1475	8	2	.1921	7	1	.0992	11	1
PGM_1	1565	10	.1564	8	.1315	9	4	.0485	16	1	.2215	6	3
C_3	.1419	11	.1095	11	.1073	12	2	.0339	18	1	.1868	7	1
Fy	.0706	12	.0673	13	.0671	15	3	.0517	15	1	.0838	12	2
Inv	.0587	13	.0554	14	.0673	14	1	.1294	10	1	...	19	0
K	.0416	14	.0164	17	.1161	11	2	.1505	8	1	.0788	14	1
ADA	.0386	15	.1072	12	.0752	13	2	.1428	9	2	.0020	16	0
AK	.0344	16	.0316	15	.0377	19	1	.0715	14	1	.0010	18	0
P	.0298	17	.0311	16	.0484	16	2	.0372	17	1	.0605	15	1
Lu	.0274	18	.0164	18	.0387	18	1	.0735	13	1	.0010	17	0
6PGD	.0229	19	.0010	19	.0390	17	1	.0009	19	0	.0803	13	1
Tf	.0079	20	0	20	0	20	0	0	20	0	0	20	0

NOTE. —P = probability of the exclusion of a random mother-child pair. P_{ex} = average proportion of subjects excluded by observed mother-child pairs. N = no. exclusions revealed by a marker system in our sample of "nonfathers." R = row in order of decreasing efficiency of marker systems —first column, in the population; other columns, in our sample.

paternity exclusion. It would, thus, seem that chance alone explains this absence of exclusion by the Hp system.

Table 5 compares the theoretical probability of exclusion (P) by a given system to the average proportions of subjects excluded (P_{ex}) by the mother-child pair in the following samples: the 61 trios without exclusion and the 25 trios with exclusions, subdivided into the 13 T trios and the 12 F trios.

The average proportions observed are comparable to the probability of exclusion (P), and the proportions observed on the samples of the 61 nonexcluded trios are no different from those observed in the sample of 25 excluded trios. This is important because an unequal distribution of the values assumed by the excluded proportion (P_{ex}) might have been expected to divide the sample into two subgroups, one more favorable, and the other less, for the demonstration of nonpaternity of the putative father. Among the 25 mother-child pairs that were incompatible with their putative father, however, there exists a decreased average value of P_{ex} by Hp, where few exclusions occur, and a notable rise in the average of P_{ex} for K due to a mother-child pair excluding 97.8% of the subjects (mother K−k+Kp[a−b+], child K−k+Kp[a+b+]).

The columns headed by R in table 5 indicate the theoretical ranking of systems according to their efficiency in exclusion (i.e., p_i plus the observed ranking of the same systems according to the average proportion of nonpaternity they determine in each of the four samples).

Number of Exclusions Observed and Proportion of Males Excluded by Mother and Child

For each trio giving rise to paternity exclusion, the proportion of males excluded (P_{ex}) by both the mother and child for each marker system separately and for the entire set of 20 was calculated. Total P_{ex} is .8052–.9999 (median = .9717, average = .9537) for the T trios, and .8370–.9990 (median = .9537, average = .9632) for the F trios. According to the examination procedure used, the overall probability of exclusion (P) is .9440 (tables 1 and 2).

No relationship can be demonstrated between the total P_{ex} by a trio and the number of systems showing actual exclusions; the rank correlation coefficient of Spearman is not significant: $r_s = .2975$, $z = 1.457$. This lack of relationship may be due, in the first place, to the small range of possibilities for the number of systems showing exclusion which only vary from 1 to 4 and, in the second place, to the fact that the number of exclusions observed depends on the phenotype of the putative father—in other words, to chance.

Therefore, the number of marker systems by which a mother-child pair eliminates a putative but not biological father is not only related to the overall P_{ex}, but also to the strong effect of a few systems specific to that mother-child pair and which tend to exclude a very high proportion of subjects. This hypothesis is supported by histograms of the P_{ex} values observed for each mother-child pair in the 20 systems, which show that the exclusions demonstrated involve mainly the systems responsible for a high proportion of exclusions of males respecting this particular mother-child couple [9].

Rank in Examination Sequence of the First Exclusion Observed for a Trio

Table 6 shows how many times the first exclusion of a trio is observed, according to the sequence of application of the tests. The advantage of the efficiency sequence is clear, since the four most discriminating examinations (MNSs, Rh, AcP, and Gm) disclose 19 out of 25 putative but not biological fathers when the two rules are taken into account. If the first rule of exclusion only is considered, the examination sequence must be followed far more closely: these four discriminating systems disclosed only ten putative but not biological fathers out of 25. Even the last five systems are useful (Tf having been eliminated), since each provides an exclusion for the first time on a trio according to the first rule. This discrepancy between the expected efficiency of the sequence and the rank of appearance of the first exclusion according to the first rule should not be unexpected, since the efficiency sequence has been determined from the expected exclusions according to either the first or second rule.

DISCUSSION

The most efficient sequence for excluding paternity, calculated from Mendelian laws and Western European gene frequencies is very different from the historic sequence used in the laboratory. It is the polymorphic enzymatic systems which are the most efficient because they have several alleles of high frequencies and do not show dominance, while silent alleles are almost absent.

The transfusion sequence eliminates only one out of two putative fathers who are not biological fathers; in other words, some of the antigens commonly used in transfusion typing (K, Fy^a, Jk^a, and S) are not useful for the validation of paternity.

The number of exclusions observed for each system in our sample of 171 families is in complete agreement with the efficiency sequence order (tables 4 and 5). MNSs

TABLE 6

FIRST EXCLUSIONS OBSERVED ON 25 NONFATHERS

FIRST OR SECOND RULES		FIRST RULE ONLY	
Genetic system	No.	Genetic system	No.
MNSs	10	MNSs	2
Rh	3	Rh	4
AcP	4	AcP	2
Gm	2	Gm	2
GPT	1	GPT	1
Jk	0	Jk	2
Hp	1	Hp	1
ABO	1	ABO	1
Gc	0	Gc	0
PGM_1	1	PGM_1	2
C_3	0	C_3	0
Fy	1	Fy	2
Inv	0	Inv	1
K	1	K	1
		ADA	1
		AK	1
		P	1

determines 10 exclusions, and the five systems, Rh, AcP, Gm, GPT, and Jk, each determines five. The decrease in the number of exclusions obtained by ABO and Hp must be due to a sampling bias for ABO and to chance for Hp.

The average proportion of males excluded by a given system observed among the 61 mother-child pairs not having excluded the putative father on the one hand, and among the 25 mother-child pairs incompatible with the putative father on the other, does not vary from mathematical expectations (p_i). This again confirms the importance of the efficiency sequence established on the basis of the values of p_i.

Alternatively, the number of exclusions detected respecting a putative but not biological father by a mother-child pair does not depend on the overall value of P_{ex} for the mother-child pair, but on the presence of a few elevated values of p_i' in one or several systems. Additionally, the phenotype of the excluded putative father plays a role in the number of exclusions detected and is completely random.

In practice, the use of the efficiency sequence should reduce the number of systems examined, particularly in genetic research where, in the validation of the family file, the first ten systems excluded 91% of the putative but not biological fathers.

In forensic medicine, the efficiency sequence can also be used, but usually an expert prefers to base his or her conclusion on the exclusions observed on several systems, including at least one where the first rule applies.

Table 6 shows that the first observed exclusion on each of 25 putative but not biological fathers requires 14 examinations if the first or second rule is followed, and 17 if at least one exclusion by the first rule is regarded as mandatory. Nevertheless, laboratories doing several analyses a day can work by ranked levels of five examinations, giving a rather rapid exclusion of certain families and, thus, economizing on antisera and other materials.

Finally, as has been shown [9, 10], when there is no proof of exclusion, the proportion of excluded subjects (P_{ex}) is a useful parameter for the study of such problems as unsuspected consanguinity and the quality of the laboratory examinations. If P_{ex} is low, some doubt is cast on the absence of exclusion because of such considerations.

ACKNOWLEDGMENTS

We are indebted to Dr. Claude Chastang (U 88) for writing the algorithm and the FORTRAN IV program, to Geneviève Liberge and Geneviève Jusczack (CNTS) for the blood groups phenotyping, and to Dr. Jean-Yves Muller (CNTS) for the HLA typing.

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